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Thyroid hormone influence on oxygen consumption rates, body mass, and lipid metabolism in mice with noninsulin dependent diabetes mellitus

Catherine Renee Clark

THYROID HORMONE INFLUENCE ON OXYGEN CONSUMPTION RATES, BODY MASS, AND LIPID METABOLISM IN MICE WITH NONINSULIN DEPENDENT DIABETES MELLITUS

A Thesis

Presented to the

Faculty of

California State University,

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in

Biology

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ABSTRACT

The effects of thyroid hormone on oxygen consumption rates, body mass, and lipid metabolism in C57BL/KsJ diabetic and normal mice were studied in animals treated with 0, 100, 200, or 500 ng T_3/g body weight (BW) for nine days. Baseline total oxygen consumption rates of diabetic mice were 28% greater than normal mice while, after receiving 500 ng T_3/g BW for 10 days, the rates of diabetic animals were 41% greater than normal mice. The maximal increase in specific oxygen consumption rates was found to be 20% for normal mice and 52% for diabetic mice. While baseline specific oxygen consumption rates of diabetic mice were 39% lower than normal animals, T_3-stimulated diabetic mice had rates that were only 19% lower than normal. Untreated and vehicle-treated control normal mice show a decrease in specific oxygen consumption rates with increasing body mass while no correlation between these parameters was found in diabetic mice. Following treatment with 500 ng T_3/g BW for nine days both normal and diabetic mice exhibited specific oxygen consumption rates that decreased with increasing body mass. These results were intermediary to what was seen without T_3 and both normal and diabetic mice now showed similar scaling relationships. This suggests that differences in specific oxygen consumption rates are due to T_3-responsive elements.

Lipid metabolism was also evaluated and diabetic mice showed a dose-dependent reduction in serum triglyceride concentrations while no decrease was exhibited by the normal animals. While VLDL and LDL were not quantified their concentrations appeared to remain relatively constant in all study groups. Serum HDL concentrations of the diabetic animals, however, were significantly greater than those of the normal animals at

all doses of T_3 and the concentration of HDL declined linearly with increasing doses of T_3. Diabetic animals exhibited a dose-dependent reduction in body mass while normal animals showed no loss.

This project showed that diabetic mice are relatively resistant to T_3. Differences in specific oxygen consumption rates between untreated normal and diabetic mice are not due to a metabolic scaling phenomenon. It appears that a T_3-responsive element, probably related to the lean body mass or metabolically inactive adipose tissue, is responsible for the differences observed in specific oxygen consumption rates between normal and diabetic mice. While T_3 induced a dose-dependent decrease in body mass in diabetic mice, the decline in serum triglyceride and HDL concentrations suggests reduced secretion of lipoproteins. The body mass loss, however, likely reflects loss of fat deposits from adipocytes due to increased fatty acid mobilization in response to increased energy consumption under the influence of T_3 stimulation.

ACKNOWLEDGMENTS

This project would not have been possible without the assistance of many people and, though it is impossible to thank everyone, there are a few people that I would like to recognize. I sincerely thank my graduate committee Dr. Richard Fehn, Dr. Dave Polcyn, and Dr. Klaus Brasch for their invaluable assistance with project guidance and manuscript preparation. Special thanks to my major advisor, Dr. Richard Fehn, who was instrumental in getting me into the masters program and, with a lot of patience and understanding, seeing me through to the end. I am grateful for his guidance, inspiration, encouragement, and, most of all, his friendship.

Without the aid of Cindy Chrisler, who ordered and cared for the mice, this project may never have occurred. Dwight Gallo and Mike Mahoney were of great help in ensuring that supplies and equipment were present and in working order. I can not thank Carol Smith enough for allowing me to use the department computer and for assisting in many other ways. In addition, Loma Linda University Medical Center Clinical Laboratory played a key role in this project by performing the lipoprotein electrophoresis.

My parents and brother deserve very special thanks for supporting me financially and emotionally throughout my education. Without their love, support, and understanding this project as well as my education would have been a dream and not a reality. Thank you very much. I owe a huge debt of gratitude to these individuals and many others without whose assistance this project would never have succeeded.

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CHAPTER ONE: INTRODUCTION

Diabetes mellitus is a genetically determined disease of glucose tolerance and handling which affects 5-6% of the United States population and is one of the leading causes of death and disability in the United States (1). In addition, diabetes exacts a high toll in terms of financial costs by consuming one in seven health care dollars (estimated at $105.2 billion per year) (2) due to frequent physician and hospital visits. Diabetes mellitus is broadly classified as either insulin dependent diabetes mellitus or noninsulin dependent diabetes mellitus (shown schematically in Appendix I). Insulin dependent diabetes mellitus (IDDM), also known as juvenile diabetes, is the form familiar to most people. IDDM is characterized by endogenous insulin levels that are negligible or absent due to pancreatic hyposecretion and is the type of diabetes seen most often in children and young adults although, the onset of the disease can occur at any age. Individuals with IDDM require exogenous insulin to control glucose levels (1).

Noninsulin dependent diabetes mellitus (NIDDM) or adult onset diabetes accounts for 85% of the cases of diabetes mellitus. NIDDM is characterized by normal to high serum levels of endogenous insulin and tissue insulin resistance. The onset of NIDDM occurs at any age but increases in prevalence with age. While a majority of individuals with NIDDM are also obese (1), the physiological basis of the obesity is unknown (3) and is the focus of this study.

The C57BL/KsJ db/db diabetic mouse serves as a model for NIDDM. Diabetic mice exhibit age-dependent onset of the syndrome which is characterized by hyperglycemia, hyperinsulinemia, insulin resistance, and obesity (4-7). NIDDM in diabetic mice, as in

humans, is genetically determined (1,7,8), yet the gene defect and mechanism(s) of the pathology remain undefined. Proposed defects in thyroid hormone responsiveness and abnormal thyroid hormone profiles make this an interesting model with which to investigate potential endocrine defects which may contribute to the obesity associated with the development of the disease.

Thyroid hormones have been shown to play major roles in regulation of development, growth, and metabolism (9,10). Thyroxine (T_4), a hormone that is synthesized and secreted by the thyroid gland, is converted by the liver and kidney to a metabolically active form of the hormone triiodothyronine (T_3). The actions of thyroid hormones are mediated through the binding of the T_3 to nuclear receptors located on the DNA. The receptors are protein complexes which bind specific regions of DNA to regulate gene transcription and thus, the expression of gene products (9). The regulation of thyroid hormone-responsive genes can occur through control of gene transcription rates, the stability of the mRNA produced, and the rate of degradation of the protein products (10). It has been suggested that thyroid hormones exhibit tissue-specific regulation at all of these levels (9).

Defects in thyroid hormone responsiveness have been demonstrated in the diabetic mouse model for NIDDM. Obese-diabetic animals exhibit higher serum T_3 concentrations (11,12) and lower metabolic responses to T_3 (13) when compared to their normal counterparts. Previous studies in our laboratory (personal communication) have shown that diabetic mice have a relative insensitivity to T_3 in that, they do not respond metabolically to physiologic concentrations of T_3 but remain capable of responding to supraphysiological concentrations of T_3 (Figure 1). The reason for the failure of the

diabetic mice to respond to physiological concentrations of T_3 remains unknown. However, previous studies have shown that the binding affinity of the hepatic T_3 receptors is decreased in stable obese diabetic animals, 13-25 weeks of age, when compared to their normal counterparts (Figure 2). Since the nuclear T_3 receptors of diabetic animals are not able to bind T_3 as tightly as those of normal animals the binding differences could lead to attenuated responses due to shorter binding times and reduced gene regulation. In addition, the number of T_3 receptors is increased in obese diabetic animals when compared to the normal mice and, though the total number of binding sites is greater than normal, a defect remains which prevents the diabetic animals from responding to normal serum concentrations of T_3. The decreased binding affinity, defective receptors, and/or a post-receptor defect likely contribute to the decreased metabolic response to T_3, thus normal T_3-regulated processes are compromised in diabetic mice.

Thyroid hormone has been shown to be an important regulator of intermediary metabolism. Both hepatic lipogenesis and adipocyte lipolysis are stimulated by T_3. T_3 stimulates an increase in the transcription of mRNA for lipogenic enzymes such as malic enzyme, glucose 6-phosphatase, and fatty acid synthase (9,14). Lipolysis in adipocytes is also stimulated by T_3 and this leads to the breakdown of lipids and release of fatty acids which may be used as an energy source for peripheral tissues (15). A synergistic hormone, growth hormone (GH), is involved in the lipolytic responses and is also dependent upon control by T_3. T_3 leads to an increase in the synthesis and secretion of pituitary growth hormone (GH) (16,17). Growth hormone, in turn, plays a permissive role to T_3-induced lipolysis in adipocytes (18). Paradoxically, while T_3 induces hepatic

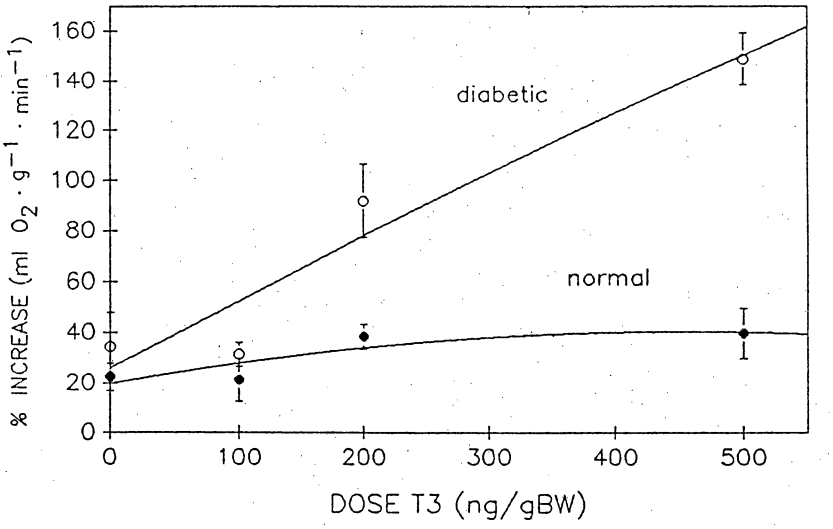


Figure 1. Dose-response curve for relative increases in specific oxygen consumption rates of normal (●-●) and diabetic (0-0) mice treated on nine consecutive days with varying doses of T_3. Diabetic animals attain a maximal response at 500 ng T_3/g BW that was 150% above basal levels while the normal animals attain a maximal response at 200 ng T_3/g BW (40% above basal) and show no further increase. Values are mean ± SEM, n=3 (Fehn and Lang, unpublished observations).

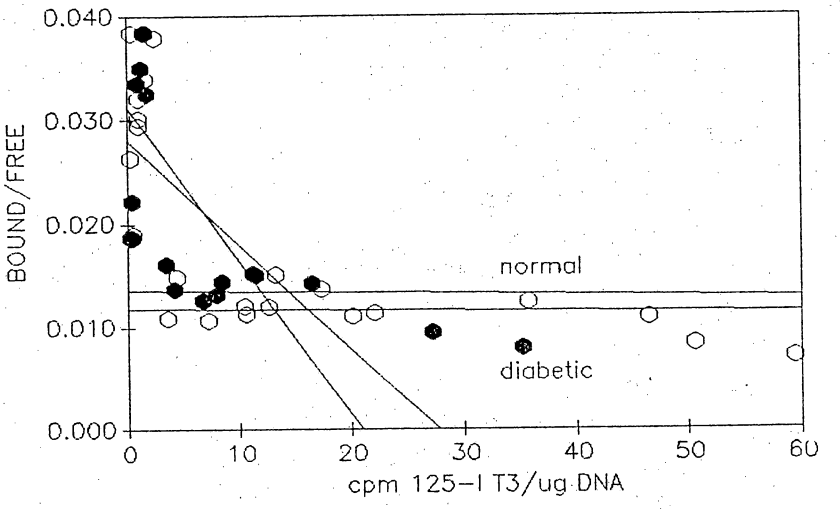


Figure 2. Scatchard plot of hepatic T_3 receptors of normal (0-0) and diabetic (●-●) mice where the intercept with the abcissa indicates the approximate number of binding sites and the slope of the line indicates the binding affinity. Diabetic mice have a decreased binding affinity for T_3 and a greater number of binding sites in the hepatocyte compared to normal mice.

lipogenesis it also induces the growth hormone which is necessary to allow adipocytes to respond to T_3-induced lipolysis (shown schematically in Appendix II). Thus, T_3 plays a role in both lipogenesis and lipolysis.

Alterations in lipid metabolism have been demonstrated in the C57BL/KsJ diabetic mouse. Diabetic mice have been shown to have high serum triglyceride and cholesterol levels. It has been hypothesized that the elevated triglyceride levels are due to increased secretion of triglycerides from the liver and/or a defect in the clearance of triglycerides from the blood. In addition, it has been proposed that the basal rate of lipolysis in adipocytes of diabetic animals is increased (8).

Part of the altered lipid metabolism seen in diabetic mice may result from attenuated T_3-induced, GH-dependent lipolysis in adipocytes which results from suppression of pituitary GH synthesis (19) and secretion due to the increased concentrations of insulin normally present in these animals. Prager et al (16) have demonstrated that insulin suppresses T_3-stimulated GH mRNA levels in vitro. Suppression of GH was apparent 24 hours after the addition of supraphysiological concentrations of insulin to rat pituitary tumor cells and did not decrease until 96 hours following the removal of the insulin (16). Since GH is necessary to prime the adipocyte for T_3-responsive lipolysis to occur, the blunting of this response by hyperinsulinemia may partially account for the obesity seen in diabetic mice. If the rates of hepatic lipogenesis and storage of lipids in adipocytes remain normal while the release of lipids from adipocytes is decreased due to GH deficiency-induced thyroid hormone-insensitivity this could lead to net fat deposition and contribute to the development of obesity. Tissue-specific alterations in T_3-responsiveness therefore,

could help explain this phenomenon.

This research project was designed to study, in diabetic mice, metabolic responses to T_3 by measuring oxygen consumption rates as an indicator of total energy metabolism and by evaluating the effects of T_3 treatment on lipid metabolism through the analysis of serum triglyceride and serum very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) levels. In vivo oxygen consumption using a flow-through oxygen analyzer was also employed because it offers a convenient and noninvasive technique for measuring metabolic rates in intact animals.

Since T_3 plays a major role in stimulating metabolism, increased levels of T_3 would be expected to lead to increased energy utilization and hence increased oxygen consumption rates, as has been demonstrated previously in obese mice (20). The increased metabolic rates should reflect the increased demands for energy, which may be met by available glucose via glycolysis and in subsequent long-term demands by glycogenolysis. When the glycogen stores are depleted however, a conversion to lipid metabolism should occur. VLDL serves to transport lipids from the liver to other tissues and, thus, the levels of serum VLDL would be expected to increase under increased energy demand. However, it is important to note that as the VLDL releases its lipids it is converted into LDL which may cause serum VLDL concentrations to decrease. Most likely the serum LDL concentrations would be expected to decrease since LDLs bind to receptors and are then internalized and degraded by muscle, mammary, adipose, and steroidogenic tissues to supply cells with cholesterol (21). In addition, serum HDL concentrations would be expected to decrease or remain stable since HDL does not serve as a source of fuel but

rather as a cholesterol transport molecule.

It is the intent of this study to compare T_3-associated metabolic responses in normal and diabetic mice to determine whether there are interphenotypic differences and to determine if T_3 treatment leads to intraphenotypic changes in lipid metabolism.

CHAPTER TWO: MATERIALS AND METHODS

Animals

C57BL/KsJ homozygous db/db diabetic and heterozygous db/m normal mice were obtained from the Jackson Laboratories (Bar Harbor, Maine). The animals were housed at 23 C, 40-60% humidity, and 14L:10D lighting, lights were on from 0600 to 2000 hours, in shoebox cages containing hardwood chip bedding. Tek Lab 4% rodent chow and water were provided ad libitum.

Eight- to ten-week old animals received daily intraperitoneal injections of L-3,5,3'triiodothyronine, T_3 (Sigma Chemical Company #T2877) in the following doses: 100, 200, and 500 ng/g body weight. The T_3 was prepared daily using 100 ug/ml in 0.5 mM NaOH. Control animals received intraperitoneal injections of vehicle (0.5 mM NaOH) in proportional volumes (2 ul/g BW). Each experimental group consisted of five db/db diabetic and five db/m normal animals. Injections were given between 0800 and 1200 hours for nine consecutive days (day 1 through 9) following metabolic readings (see below).

Oxygen Consumption

The oxygen consumption of each experimental animal was measured by an Amtek

S-3A/II Oxygen Analyzer at a flow rate of 140 ml per minute (as determined by Gilmont Flowmeter D-665). Ten minute readings were taken on all animals on days 1 through 10 between 0700 and 1145 hours. Animals were weighed daily for specific oxygen consumption determinations and possible weight changes. Total in vivo metabolic responses to T_3 were reported as total oxygen consumption (ml $O_2 \cdot min^{-1}$), specific oxygen consumption (ml $O_2 \cdot g^{-1} \cdot min^{-1}$), and percent increase in specific oxygen consumption above baseline levels. All animals were evaluated in the nonfasting state. The data were subjected to analysis of variance (22) and compared by Duncan's Multiple Range Test (23).

Blood Collection and Analysis

All mice were sacrificed by CO_2 euthanasia on day 10 starting at 1330 hours. Blood was collected by cardiac puncture using a 23 gauge needle on a 1.0 ml syringe. Samples were placed in microtainer serum separator tubes (Becton Dickinson) and then refrigerated at 4 C until clotted. The samples were then centrifuged at 1700 x g for 40 minutes at 4 C. Recentrifugation was conducted on samples when necessary. The serum was aliquoted into 1.5 ml microcentrifuge tubes in the following manner: 100 ul for serum HDL determinations and 20 ul for serum triglyceride determinations. All samples were stored at 4 C until assayed.

Serum concentrations of VLDL, LDL, and HDL were determined using the REP Ultra-30 HDL, VLDL/LDL Cholesterol electrophoresis system. This system uses an agarose gel to separate serum lipoproteins by molecular weight. The amount of cholesterol and cholesterol esters present in the sample are directly proportional to the

amount of quinoneimine dye which is used to visualize the band and is produced through enzymatic degradation by cholesterol esterase (24). The electrophoresis was performed by Loma Linda University Medical Center Clinical Laboratory. Relative quantitation of the samples, using a photoimage of the gel, was performed using a BIORAD model 620 Video Densitometer. Data are reported in optical density units.

Serum triglyceride levels were determined by commercial assay (Sigma Chemical Company #339) using a colorimetric determination involving enzymatic degradation by lipoprotein lipase. Percent transmittance was read at 550 nm on a microtiter plate reader (BioKinetics Reader EL312e). Data are reported as mg/dL.

Data were subjected to analysis of variance and compared by Duncan's Multiple Range Test.

Percent Body Fat

Body weights of the dead mice were measured in air and in water using a Scientech 5500 top loading balance. Mice were suspended from an alligator clip for measurements in air. The suspended mice were then submerged in a 1L beaker containing water, massaged to release air trapped in their lungs and fur, and weighed again. The temperature of the water was recorded for water density corrections.

The Siri equation (25) was used to calculate the density of the mouse body:

$$D_b = \frac{M_a \times D_w}{M_a - M_w - RV \times D_w}$$

where:

D_w = density of the water (corrected for temperature)

M_a = mass of mouse in air

M_w = mass of mouse in water

RV = residual lung volume

The value used for the RV was 0.5 ml as determined by Rudrich and Fehn (26). The percent body fat was calculated as (25):

$$\% \text{ body fat} = \frac{495}{D_b} - 450$$

The data were subjected to analysis of variance and compared by Duncan's Multiple Range Test.

Characterization of Hepatic Thyroid Hormone Receptors

The livers of both diabetic and normal mice were excised following CO_2 euthanasia and the nuclei were isolated by homogenizing the liver in STM (0.32 M sucrose, 3 mM $MgCl_2$, 0.5% Tween). The samples were then centrifuged 10 minutes at 800 x g at 4 C. Samples were aspirated to remove the supernatant. Nuclear pellets were washed in STM, centrifuged, and aspirated two times. The nuclei were then resuspended in SM (0.32 M sucrose, 3 mM $MgCl_2$). The nuclei were divided into ten samples. Five concentrations of I-125 labeled T_3 (NEN Research Products, 1200 uCi/ug) (2.2×10^{-9}, 6.6×10^{-10},

6.6×10^{-11}, 6.6×10^{-12}, 6.6×10^{-13} M in 400 mM KCl) were added to these preparations. The samples were incubated overnight at 4 C to permit hormone-receptor complex formation. Supernatants were aspirated and the amount of T_3 bound to the receptors was determined using a scintillation counter (Picker Pace-1). The amount of DNA present in each sample was determined using the diphenylamine assay (27). The data obtained were used to generate Scatchard plots (28). Tissue samples were grouped into young (5-6 weeks of age; n=2), stable (13-25 weeks of age; n=4 for normal and n=5 for diabetic mice), and old (54 weeks of age; n=1). Data were subjected to analysis of variance and Duncan's Multiple range test.

CHAPTER THREE: RESULTS

The metabolic responses following nine days of treatment with various doses of T_3 are shown in Figure 3. Normal animals receiving 500 ng T_3/g BW exhibited a maximal increase of 20% in specific oxygen consumption above vehicle-treated normal control animals that received 0 ng T_3/g BW. The maximal increase in specific oxygen consumption for the diabetic animals receiving 500 ng T_3/g BW was 52% greater than vehicle-treated diabetic control animals. The percent increase in specific oxygen consumption rates in response to the maximum dose of T_3 is therefore 2.6 times greater in diabetic than in nondiabetic mice.

The changes in total oxygen consumption over the ten days of observation are shown in Appendix III and graphically in Figure 4. In both the normal and diabetic animals, total oxygen consumption rates were maximal on day 6, increasing 64% and 47% above

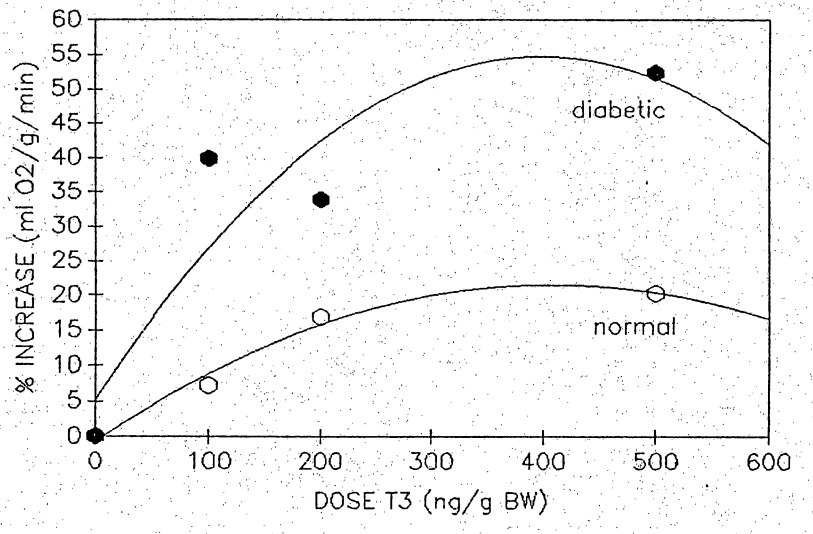


Figure 3. Dose-response curve for relative increases in specific oxygen consumption rates of normal (0-0) and diabetic (●-●) mice treated for nine days with varying doses of T_3. Diabetic animals show a maximal increase of 52% while the maximal increase in normal animals was 20%. Values are mean - mean of vehicle-treated control animals, n=5.

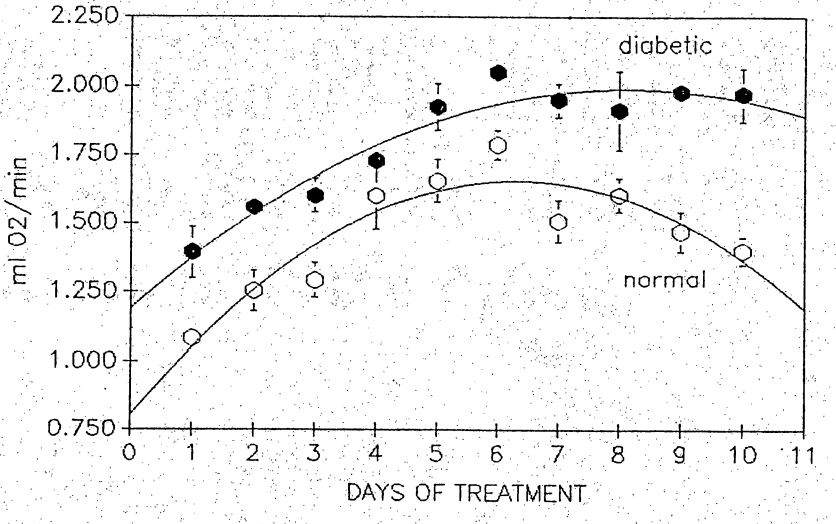


Figure 4. Daily total oxygen consumption rates of normal (0-0) and diabetic (●-●) mice during nine days of treatment with 500 ng T_3/g BW. Day one represents baseline levels prior to initial injections after which treatments were administered daily. Baseline total oxygen consumption rates of diabetic animals are 28% greater than normal animals. Diabetic mice attain rates that are 41% greater than normal animals on day 10. Diabetic animals plateau at day 6 while the normal animals attain maximal rates on day 6 and then decline. Values are mean $\pm$ SEM, n=5.

baseline levels, respectively. Each was followed by a gradual decline such that, on the tenth day of the experiment, oxygen consumption rates were only 29% and 41% above the baseline levels (day 1) for normal and diabetic animals, respectively. The diabetic animals consistently had greater total oxygen consumption rates than the normal animals, as might be expected since the diabetic animals are approximately twice the body mass of normal animals.

In contrast, the mass-specific metabolic rates show that obese-diabetic animals consumed 39% less oxygen per gram of tissue than the normal animals on the tenth day (Appendix IV and shown graphically in Figure 5). The specific oxygen consumption rates for normal animals rose from 0.049 ml $O_2 \cdot g^{-1} \cdot min^{-1}$ to a maximum of 0.082 ml $O_2 \cdot g^{-1} \cdot min^{-1}$ at day 6 and then declined to 0.062 ml $O_2 \cdot g^{-1} \cdot min^{-1}$ by day 10. The diabetic animals however, showed consistent daily increases in specific oxygen consumption rates from 0.030 ml $O_2 \cdot g^{-1} \cdot min^{-1}$ to 0.050 ml $O_2 \cdot g^{-1} \cdot min^{-1}$ over the full ten days of study.

Evaluation of the dose-dependent responses to T_3 induction of metabolism using total (Figure 6) and specific (Figure 7) oxygen consumption rates for the normal animals on day 6 of the experiment showed that rates did not change significantly from 0 ng T_3/g BW to 100 ng T_3/g BW nor from 0 ng T_3/g BW to 200 ng T_3/g BW dose. The total and specific oxygen consumption rates for normal mice receiving 500 ng T_3/g BW however, increased ($p \leq 0.01$) above that seen in the 0, 100 , and 200 ng T_3/g BW animals. When diabetic animals receiving 0 ng T_3/g BW were compared to T_3-treated diabetic animals, initial increases in total and specific oxygen consumption rates were evident at 100 ng T_3/g BW

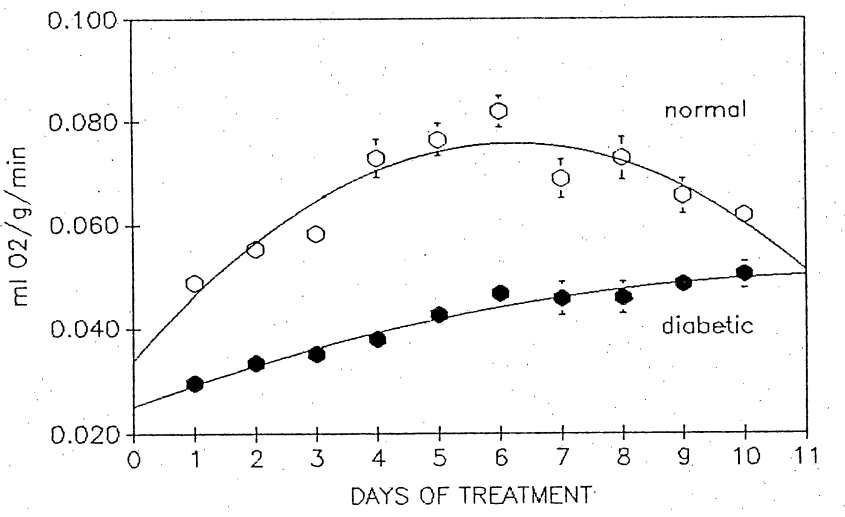


Figure 5. Specific oxygen consumption rates of normal (0-0) and diabetic (●-●) mice receiving daily injections of T_3 (500 ng/g BW) over a ten day period. Day one represents baseline rates prior to initial injections after which treatments were administered daily. Baseline specific oxygen consumption rates of diabetic animals are 39% lower than normal animals. On day 6 the specific oxygen consumption rates of diabetic animals are 43% less than normal while on day 10 the rates of diabetic animals are only 19% less than normal animals. Diabetic animals show a linear increase in specific oxygen consumption rates while the normal animals attain maximal rates on day 6 and then decline. Values are mean $\pm$ SEM, n=5.

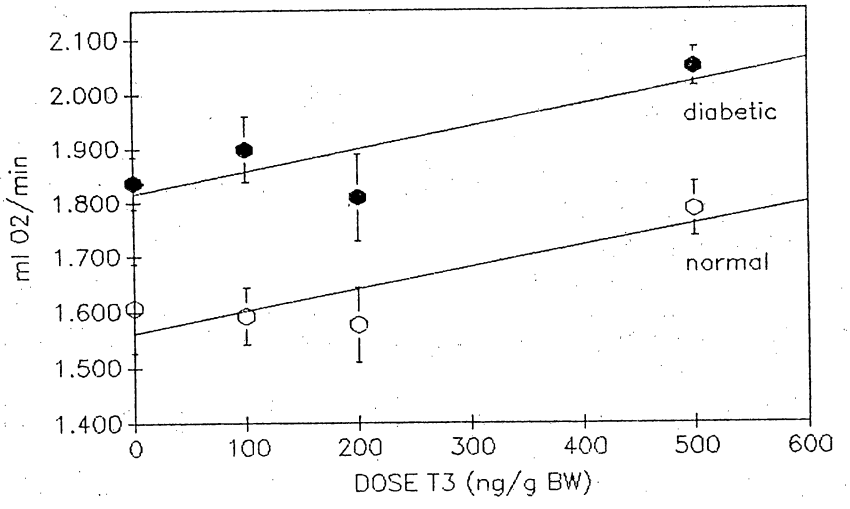


Figure 6. Dose-dependent changes in total oxygen consumption rates of normal (0-0) and diabetic (●-●) mice receiving daily injections of T_3 (0, 100, 200, or 500 ng/g BW) over a six day period. The increases are similar for both diabetic and normal mice, however the absolute values are greater for diabetic animals than for normal animals at all doses ($p \leq 0.01$). Values are mean ± SEM, n=5.

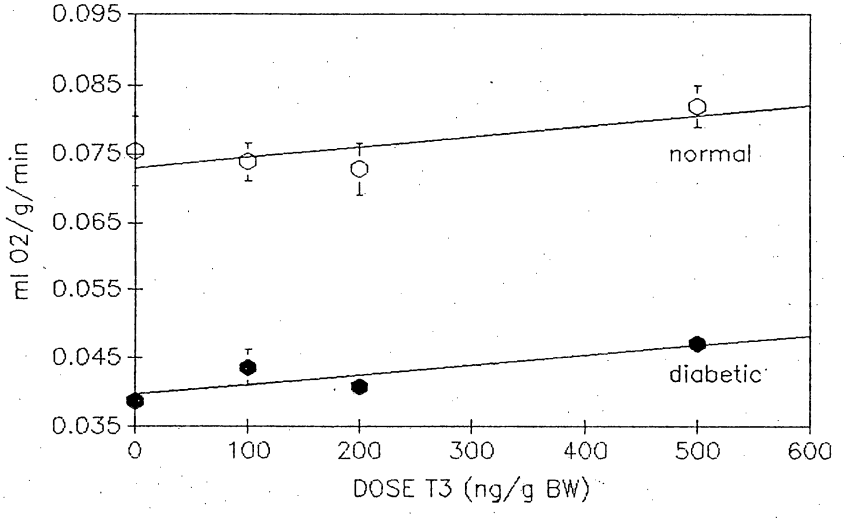


Figure 7. Dose-dependent changes in specific oxygen consumption rates of normal (0-0) and diabetic (●-●) mice receiving daily injections of T_3 (0, 100, 200, or 500 ng/g BW) over a six day period. The increases are similar for both diabetic and normal animals, however the absolute values for the diabetic mice are lower than those of the normal animals at all doses ($p \leq 0.01$). Values are mean ± SEM, n=5.

($p \leq 0.05$), no changes were observed at 200 ng T_3/g BW, and then substantial increases occurred at 500 ng T_3/g BW ($p \leq 0.01$). It is noteworthy that while the total and specific oxygen consumption rates of diabetic and normal animals were statistically distinguishable ($p \leq 0.01$) at all doses, the slopes of the lines were similar for both groups (m=0.037 for normal and m=0.038 for diabetic; m=0.001 for normal and m=0.002 for diabetic for total and specific oxygen consumption rates, respectively). This suggests that the dose-response to T_3 is similar in both diabetic and normal mice. The set point however, is lower for specific oxygen consumption rates in diabetic mice than in normal mice.

The total and specific oxygen consumption rates for both normal and diabetic animals on the tenth day of the experiment are shown in Figures 8 and 9, respectively. The normal animals had total and specific oxygen consumption rates that were not significantly different from 0 ng T_3/g BW to 100 ng T_3/g BW. However, when compared to the 0 ng T_3/g BW treatment, the normal animals showed significant increases in both total and specific oxygen consumption rates at 200 ng T_3/g BW ($p \leq 0.05$) and at 500 ng T_3/g BW ($p \leq 0.01$). The diabetic animals, on the other hand, exhibited significant increases in total and specific oxygen consumption rates at all doses of T_3 when compared to the vehicle-treated (0 ng T_3/g BW) control diabetic animals ($p \leq 0.01$). Interestingly, in contrast to above, the slopes of the lines are no longer similar for the diabetic and normal mice. The slope of the oxygen consumption rates for the diabetic mice (m=0.090 for total and m=0.003 for specific) is greater than the slope for the normal mice (m=0.045 for total and m=0.001 for specific) suggesting that the dose-response is greater in the diabetic mice.

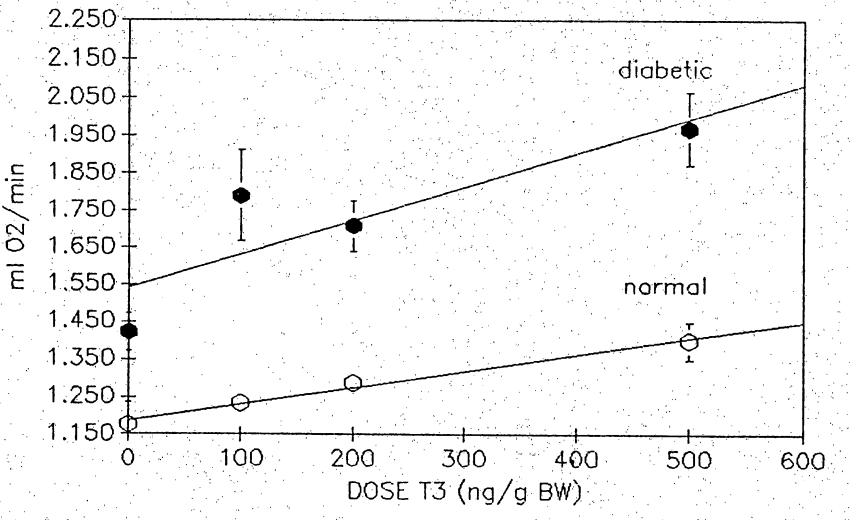


Figure 8. Dose-dependent changes in total oxygen consumption rates in normal (0-0) and diabetic (●-●) mice receiving daily injections of T_3 (0, 100, 200, or 500 ng/g BW) over a ten day period. Diabetic animals show a greater increase than normal animals ($p \leq 0.01$). Values are mean ± SEM, n=5.

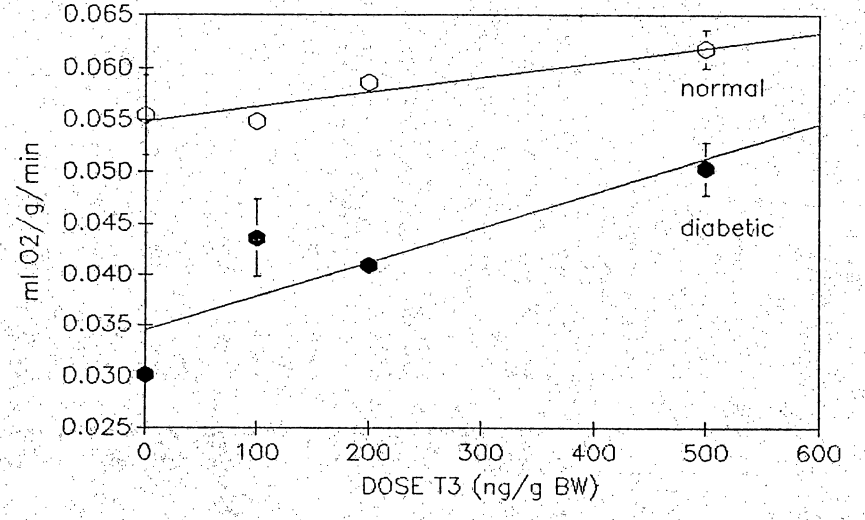


Figure 9. Dose-dependent changes in specific oxygen consumption rates in normal (0-0) and diabetic (●-●) mice receiving daily injections of T_3 (0, 100, 200, or 500 ng/g BW) over a ten day period. Diabetic animals show a greater increase than normal animals, but diabetic animals have specific oxygen consumption rates that remain lower than normal animals ($p \leq 0.01$). Values are mean ± SEM, n=5.

Normal mice showed no significant change in body mass (g) from day 1 through day 10 in any of the treatment groups (Figure 10). In contrast, the diabetic animals showed significant dose-dependent decreases in body mass at all doses of T_3 when compared to the mice receiving 0 ng T_3/g BW ($p \leq 0.01$), losing almost 17% of their initial body mass at the highest dose of T_3 (500 ng T_3/g BW).

In order to study the relationship between body mass and specific oxygen consumption rates, first order regression curves were generated for these two parameters. Normal mice, prior to the initial injections of T_3, had specific oxygen consumption rates that decreased as the body mass increased as is typically observed (m= -0.369) (23) (Figure 11). The slope of this regression was similar to that of untreated normal mice having a wider range of body masses (data not shown). In contrast, in diabetic mice, prior to initial T_3 injections, specific oxygen consumption rates were similar for all body masses (m=0.031). A lack of vehicle effect was confirmed in that normal mice receiving 0 ng T_3/g BW for ten days had specific oxygen consumption rates similar to untreated normal animals (m= -0.375) (Figure 12). The diabetic mice again did not show changes in specific oxygen consumption rates relative to body mass (m=0.013). The specific oxygen consumption rates of diabetic animals therefore, do not appear to be influenced by the body mass within the mass range studied.

To determine whether the magnitude of T_3-induced metabolic responses might be a direct function of body mass, first-order regression curves of specific oxygen consumption rates versus body mass for normal and diabetic mice receiving 500 ng T_3/g BW for ten days were generated (Figure 13). At this dose of T_3 both the normal and diabetic mice

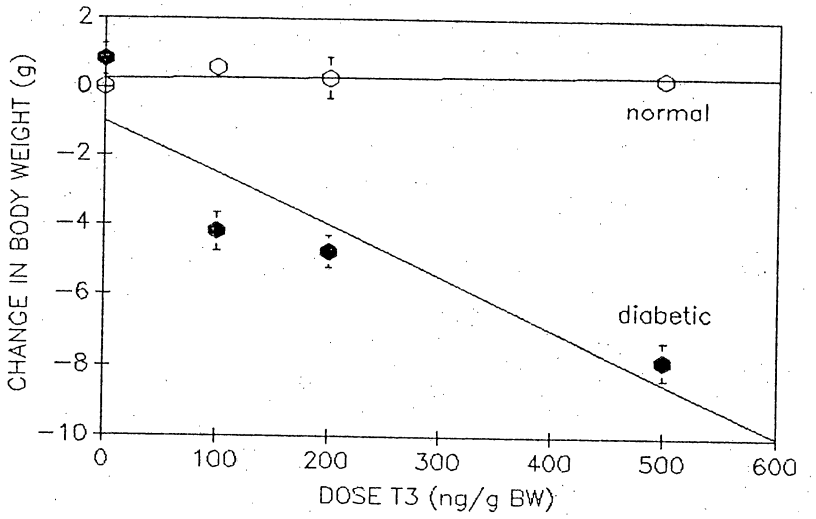


Figure 10. Dose-dependent weight loss in normal (0-0) and diabetic (●-●) mice receiving daily injections of T_3 (0, 100, 200, or 500 ng/g BW) over a ten day period. Diabetic animals show a dose-dependent reduction in body weight compared to vehicle-treated controls ($p \leq 0.01$) while normal animals show no loss ($p > 0.05$). Values are mean $\pm$ SEM, n=5.

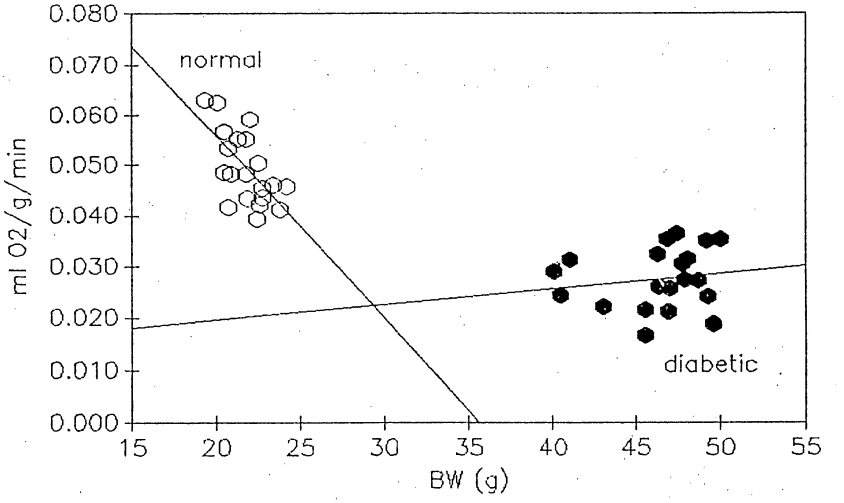


Figure 11. First order regression curve of specific oxygen consumption rates at various body masses (g) of normal (0-0) and diabetic (●-●) mice on day one prior to initial injections of T_3. Normal animals show a decrease in specific oxygen consumption rates as the body mass (g) increases. However, the diabetic animals had specific oxygen consumption rates that were not in proportion to total body mass (g).

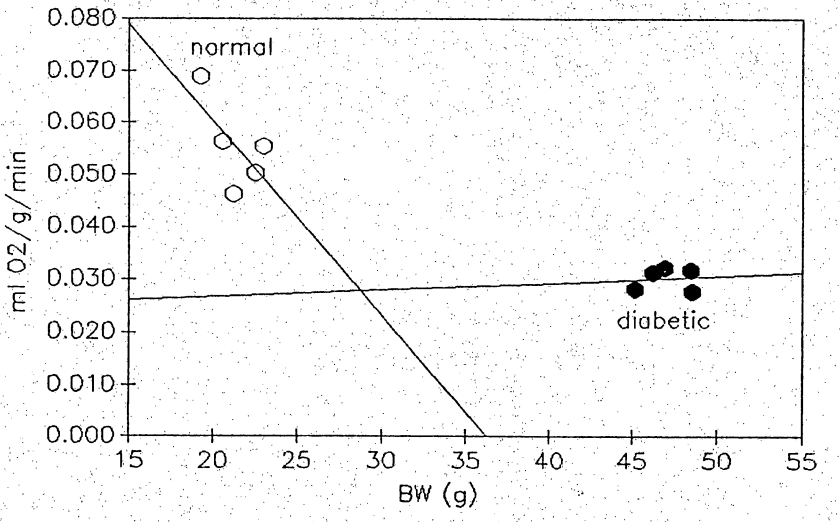


Figure 12. First order regression curve of specific oxygen consumption versus body mass (g) of normal (0-0) and diabetic (●-●) mice receiving daily injections of vehicle (2 ul/g BW) over a ten day period. Normal animals show a decrease in specific oxygen consumption rates as the body mass (g) increases. The diabetic animals however, did not show any changes in the specific oxygen consumption rates as the body mass (g) increased.

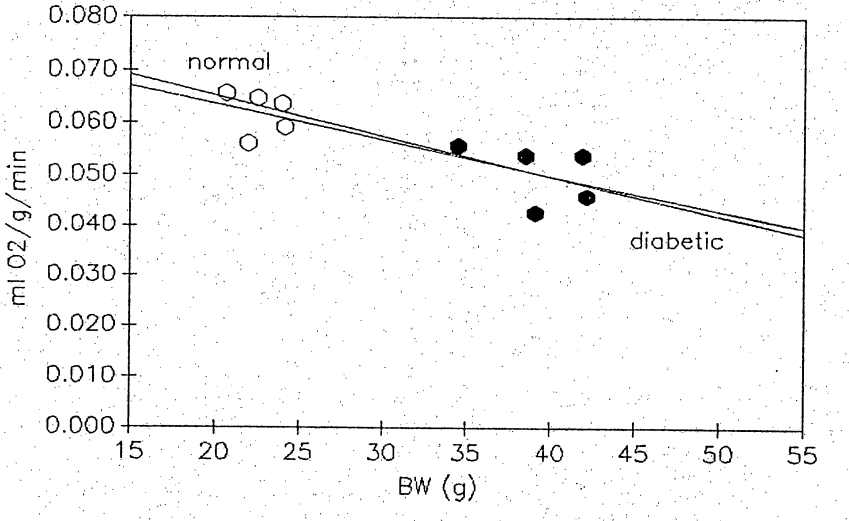


Figure 13. Regression curve of specific oxygen consumption rates at various body masses (g) of normal (0-0) and diabetic (●-●) mice receiving daily injections of 500 ng T_3/g BW over a ten day period. At this dose of T_3 the normal and diabetic animals both exhibit specific oxygen consumption rates that decrease as the body mass (g) increases. Both exhibit responses that are intermediate to untreated animals and now have the same scaling relationship.

were fully stimulated metabolically and both had specific oxygen consumption rates that decreased as the body mass increased (m= -0.067 for normal and m= -0.073 for diabetic animals). These results were intermediate to what had been observed in normal and diabetic mice receiving 0 ng T_3/g BW and both groups were now on the same scaling curve. This clearly shows that metabolic responses are different in vehicle-treated diabetic mice when compared to vehicle-treated normal mice but, are more uniform with T_3 treatment.

Serum triglyceride levels were analyzed to assess lipid mobilization in response to T_3 (Appendix V and shown graphically in Figure 14). The serum triglyceride levels in normal animals remained constant at all doses of T_3 relative to the vehicle-treated (0 ng T_3/g BW) normal control animals ($p>0.05$). Interestingly, thyroid hormone treatment significantly lowered serum triglyceride concentrations in diabetic mice at all doses of T_3 in a dose-dependent manner when compared to the vehicle-treated diabetic control animals. Diabetic animals that received T_3 at doses of 100, 200 and 500 ng/g BW had triglyceride levels that decreased by 38%, 29%, and 41%, respectively, when compared to diabetic animals receiving 0 ng T_3/g BW. The serum triglyceride levels of the diabetic mice were consistently elevated compared to those of the normal mice ($p\leq0.01$).

Serum profiles of VLDL, LDL, and HDL were attempted to assess lipid mobilization and transport (Figure 15). The gel system did not provide quantifiable images of VLDL or LDL due to very light staining of these bands. The HDL banding, however, was clear and therefore subjected to quantification by densitometry (Appendix VI and show graphically in Figure 16). The diabetic animals that received vehicle only have a

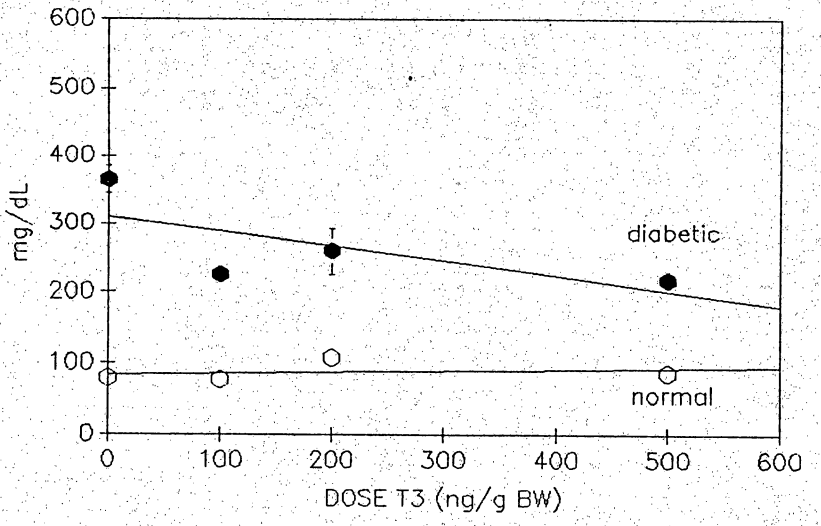


Figure 14. Regression curve of dose-dependent decrease in serum triglyceride concentration in normal (0-0) and diabetic (●-●) mice receiving daily injections of T_3 (0, 100, 200, or 500 ng/g BW) over a ten day period. Diabetic animals show a reduction in serum triglyceride concentration ($p \leq 0.01$) while normal animals show no change ($p > 0.05$). Values are mean $\pm$ SEM, n=5.

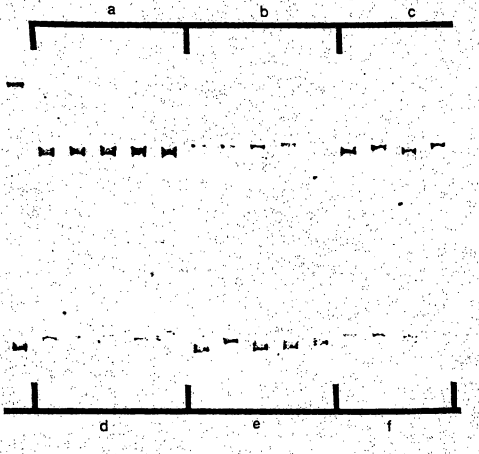


Figure 15. Agarose gel of serum lipoproteins in 75 ul serum samples collected from nonfasting normal and diabetic animals receiving varying doses of T_3 (0, 100, or 200 ng/g BW) for ten days. The samples are grouped as follows: a) diabetic 0 ng T_3/g BW; B) normal 0 ng T_3/g BW; c) diabetic 100 ng T_3/g BW; d) normal 100 ng T_3/g BW; e) diabetic 200 ng T_3/g BW; f) normal 200 ng T_3/g BW.

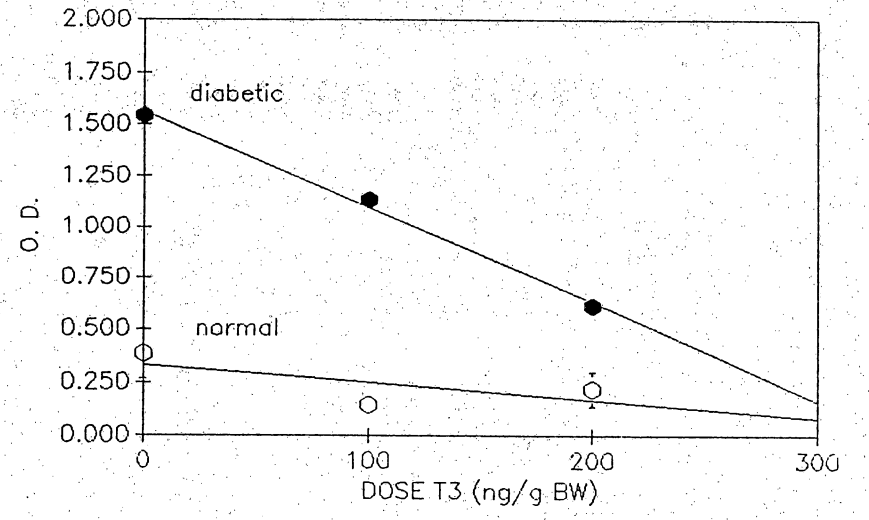


Figure 16. Regression curve of serum HDL concentration determined by desitometric scan of gel shown in Figure 15. Dose-dependent decrease in serum HDL concentration in normal (0-0) and diabetic (●-●) mice receiving daily injections of T_3 (0, 100, or 200 ng/g BW) over a ten day period. Diabetic animals show a linear decrease in serum HDL concentration ($p \leq 0.01$) while normal animals show a decrease from 0 ng T_3/g BW to 100 ng T_3/g BW ($p \leq 0.01$) and no further change from 100 ng T_3/g BW to 200 ng T_3/g BW ($p > 0.05$). The serum HDL concentrations of the diabetic animals are greater than those of the normal animals at all doses of T_3 ($p \leq 0.01$). Values are mean $\pm$ SEM, n=5 except normal 200 ng/g BW where n=4.

significantly higher concentration of serum HDL which approaches six times that of their normal counterparts ($p \leq 0.01$). A dose-dependent reduction in HDL is apparent in that normal animals exhibited a significant decrease in serum HDL concentrations from 0 ng T_3/g BW to 100 ng T_3/g BW ($p \leq 0.01$) and from 0 ng T_3/g BW to 200 ng T_3/g BW ($p \leq 0.01$), but did not exhibit a significant change from 100 ng T_3/g BW to 200 ng T_3/g BW ($p > 0.05$). A linear decrease in HDL concentrations was observed in diabetic animals with increasing doses of T_3 ($p \leq 0.01$). Although a decrease in HDL concentrations is seen in both the normal and diabetic animals at 100 and 200 ng T_3/g BW doses when compared to vehicle-treated control animals, the HDL concentrations of the diabetic animals remain greater than those of the normal animals ($p \leq 0.01$). The serum HDL concentrations of the 500 ng T_3/g BW normal and diabetic animals were not obtained.

An analysis of body composition (% body fat) was performed to determine whether increased metabolic demands were coupled with selective decreases in fat, presumed to be used as a fuel source. The percent body fat of the normal mice significantly increased at 100 ng T_3/g BW ($p \leq 0.01$) but showed no significant change at 200 ng T_3/g BW and 500 ng T_3/g BW ($p > 0.05$) when compared to the vehicle-treated control animals (Table 1). The diabetic animals, on the other hand, exhibited a significant decrease at 100 ng T_3/g BW ($p \leq 0.01$), showed no significant change at 200 ng T_3/g BW ($p > 0.05$), and again exhibited a significant decrease at 500 ng T_3/g BW ($p \leq 0.05$) when compared to vehicle-treated control animals. In normal animals, low doses of T_3 appear to either induce fat deposition or reduce lean body mass while high doses lead to no disproportionate changes in composition. Diabetic animals, on the other hand, show

Table 1. Percent body fat presented as mean ± SEM, n=5.

	0 ng T_3/g BW	100 ng T_3/g BW	200 ng T_3/g BW	500 ng T_3/g BW
NORMAL	22.70 ± 0.90	24.58 ± 1.01	23.14 ± 1.43	22.13 ± 1.01
DIABETIC	36.17 ± 1.11	34.60 ± 0.93	35.52 ± 0.20	34.91 ± 0.56

reductions in fat composition with increasing doses of T_3 except at the 200 ng T_3/g BW dose. The normal mice consistently had a lower percent body fat (23.14 $\pm$ 0.55%, n=5) than their obese-diabetic counterparts (35.30 $\pm$ 0.38%, n=5).

Assessment of hepatic T_3 receptors was performed to provide a possible explanation for thyroid hormone resistance seen in diabetic mice. The total amount of T_3 bound to the receptor was found to be age- and phenotype-dependent (Table 2). Young diabetic mice (5-6 weeks of age) had less T_3 bound to the receptors than the young normal mice. The stable diabetic mice (13-25 weeks of age) and old diabetic mice (54 weeks of age) had more T_3 bound to the receptors than their normal counterparts. The approximate maximal binding capacity (MBC) for the high affinity, low capacity (functional) receptors exhibited the same pattern with the diabetic young mice having a decreased MBC while diabetic stable mice and diabetic old mice had an increased MBC compared to their normal counterparts. The Kd, 50 percent saturation of the binding of a hormone to its receptor, was found to be just the opposite of the above. The young diabetic mice had an increased Kd while the stable and old diabetic mice had decreased Kds when compared to their normal counterparts.

CHAPTER FOUR: DISCUSSION

Previous studies in this laboratory have shown that it was possible for adult diabetic animals (30-60 weeks of age) to attain total and specific oxygen consumption rates of normal magnitude when given sufficiently high concentrations of T_3 (500 ng/g BW) for ten days. These results indicate that the T_3-responsive processes in the obese-diabetic animals

Table 2. Characterization of hepatic thyroid hormone receptors.

	NORMAL			DIABETIC		
	YOUNG (5-6 weeks of age)	STABLE (13-25 weeks of age)	OLD (54 weeks of age)	YOUNG (5-6 weeks of age)	STABLE (13-25 weeks of age)	OLD (54 weeks of age)
Total Binding (cpm/ug DNA)	3422	4612	2262	1894	6660	2454
Maximal Binding Capacity (fmol/mg DNA)	14	21	11	9	28	16
Kd (fmol/mg DNA)	7	8	6	4.5	10	9

are preserved and that diabetic animals are relatively, not absolutely, insensitive to T_3.

In the present study the young diabetic animals (8-10 weeks of age) approach, but never attain total and specific oxygen consumption rates of normal magnitude. However, the changes seen in both the normal and diabetic animals in this study were of a lesser magnitude than those observed in the older mice used in the previous study. Possible explanations for this discrepancy may be age-dependent physiological differences, changes in body composition with age or simply differences in mass. The initial masses of both the normal and diabetic adult animals used in the previous study (26.65 ± 0.63 g vs. 48.91 ± 1.72 g, respectively) were significantly higher than those of the normal and diabetic young animals of this study (21.81 ± 0.29 g vs. 46.35 ± 0.67 g, respectively; $p \leq 0.01$). If the discrepancy was due simply to mass then direct comparisons of oxygen consumption rates between age groups and phenotypes would be compromised.

Vertebrate animals have been shown to have specific oxygen consumption rates that decrease as the mass of the animal increases (29). This is typically attributed to the proportionately smaller surface area relative to body volume which reduces environmental exchanges hence, the lower energy expenditures. Diabetic mice were found to have mass-specific oxygen consumption rates that were about half that of normal animals and two possible explanations can be proposed for the differences. First, if the differences in the specific oxygen consumption rates between normal and diabetic animals were simply due to the differences in body mass and proportional surface area then, according to extrapolation from the regression curve (Figure 13), a 22 g diabetic mouse should have a predicted specific oxygen consumption rate comparable to a 22 g normal mouse. This is

not the case. Diabetic animals of this mass would have a predicted specific oxygen consumption rate of approximately 0.020 ml $O_2 \cdot g^{-1} \cdot min^{-1}$ which is clearly lower than the measured specific oxygen consumption rate of 22 g normal animals (0.050 ml $O_2 \cdot g^{-1} \cdot min^{-1}$). Thus, it appears that the differences in oxygen consumption rates are not due to a scaling phenomenon but rather an alteration in some physiological mechanism. The observation, that maximal stimulation of diabetic and normal mice (with 500 ng T_3/g BW for ten days) led to a scaling relationship in metabolic response that was intermediate to those observed in untreated animals, suggests that the mechanism responsible for the observed differences between phenotypes and ages is T_3-responsive. In addition, these results suggest that the T_3-responsive element is related to the lean body mass (30) or to the activation of previously inactive adipose tissue.

Although the changes in body mass were very similar in both old and young mice, the magnitudes of metabolic increases seen with T_3 treatment were quite different. Neither young nor old normal animals exhibited a significant change in body mass at any dose of T_3. Conversely, the adult diabetic animals had a loss in mass that approached 20% while the young diabetic animals similarly approached a 17% loss in mass at 500 ng T_3/g BW. There does not appear to be an age-dependent difference in T_3 effect on the loss of body mass. This supports the idea that the metabolic differences were not due to differences in total body mass since alterations in body mass do not occur in normal animals and occur in diabetic animals but, both exhibit increases in oxygen consumption rates with T_3 treatment. In addition, this suggests that the difference in the magnitude of increase in specific and total oxygen consumption rates observed between age groups may be due to

the ability of the animals to compensate for the increased T_3 levels from exogenous sources. Another possible explanation is, if the increased oxygen consumption rates are due to the activation of adipocytes, older animals have proportionately more fat and thus could have increased fatty acid mobilization to a greater degree in response to increased energy consumption.

The young animals in this study were at the stage in development where the serum T_3 levels are just starting to stabilize while the serum T_3 levels in the adult animals used in the previous study had been stabilized for some time (Figure 17). The T_3 resistance that is associated with NIDDM may still be developing in the young animals thus allowing them to regulate their metabolic responses to the varying doses of T_3. However, it is likely that the T_3 resistance is fully established in the older animals limiting the amount of regulation they can achieve. The differences in the levels of T_3 resistance between the old mice and the young mice may be due to changing receptor populations as has been shown in this lab (Table 2). The old diabetic mice had hepatic T_3 receptor populations that most closely resembled the normal mice and thus, could be expected to respond in a similar fashion metabolically when stimulated maximally by exogenous T_3. The young diabetic mice, on the other hand, had fewer hepatic thyroid hormone receptors than the young normal mice which could render them, even though they received the same maximum dose of T_3 as the old mice, unable to respond to the same degree. Thus, it appears that the metabolic responses to T_3 are age-dependent.

Physiological sensitivity to T_3 was evaluated by using dose-response curves of T_3-stimulated increases in oxygen consumption rates in normal and diabetic mice. The 100

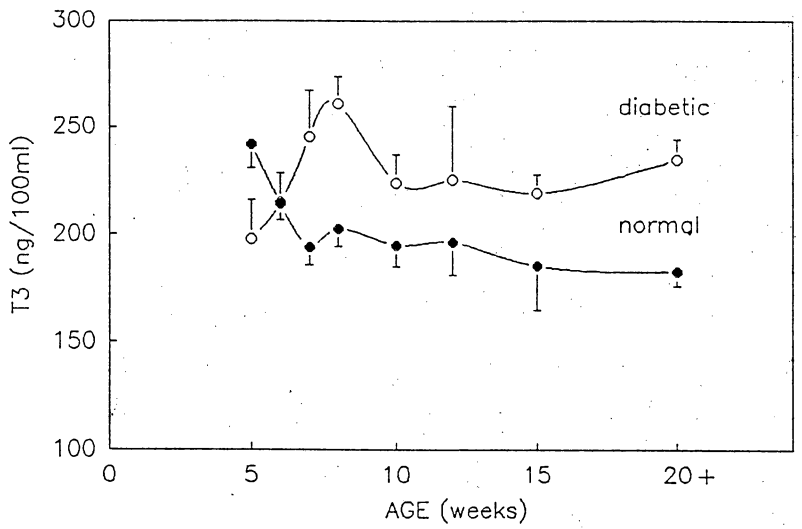


Figure 17. Nonfasting serum T_3 concentrations of normal (●-●) and diabetic (0-0) mice at various ages. The T_3 concentrations of diabetic animals (233 $\pm$ 39 ng/100 ml, n=50) are greater than those of normal animals (201 $\pm$ 40 ng/100 ml, n=127; $p \leq 0.05$). Values are mean $\pm$ SEM (19).

ng/g BW dose of T_3 represents replacement of the normal physiological concentration of T_3. Normal mice at 100 ng/g BW T_3 likely decrease the amount of endogenous T_3 produced in response to the addition of exogenous T_3 and therefore, these animals are not exposed to increased levels of T_3. The oxygen consumption rates on day 6 reflect this since they are not significantly different from the oxygen consumption rates of the vehicle-treated control animals. At the same dose and time the diabetic animals however, show an increase in oxygen consumption rates and this serves to emphasize the differential responsiveness in these animals. This may occur because the 100 ng/g BW dose of T_3 raises the already elevated levels of endogenous T_3 to achieve a supraphysiological dose allowing the animals to respond metabolically. The 200 ng/g BW dose of T_3 is the first supraphysiologic dose of T_3 used in this study. Neither normal nor diabetic animals show a significant change in oxygen consumption rates at 200 ng/g BW when compared to the animals that received 0 ng/g BW. This was an unexpected result, but it appears that both the normal and diabetic animals undergo a rebound phenomenon in responsiveness and therefore, no changes occur in the oxygen consumption rates. This rebound phenomenon may be due to down regulation of T_3 receptor populations and/or decreased levels of thyroid stimulating hormone (TSH) which would lead to decreased production of endogenous thyroid hormones. Brent et al (31) have shown that the maximum reduction in receptors is 20% thus, down regulation of thyroid hormone receptors by itself can not explain the rebound phenomenon. Another possible explanation for the unchanged oxygen consumption rates is that increased clearance of the T_3 from the serum could prevent a supraphysiological dose from occurring. However, it is likely that the 500

ng/g BW dose of T_3 is so high that the animals are not able to compensate (or compensate to a much lesser degree) and an increase in oxygen consumption is observed. The 500 ng /g BW dose of T_3 is a pharmacologic dose which pushes the limits of the physiological scope of responses.

The oxygen consumption rates for normal and diabetic mice were found to be time dependent in that the rates were different on day 6 than they were on day 10. On day 6 no change was observed in total and specific oxygen consumption rates of normal and diabetic animals at the 200 ng T_3/g BW dose. However, on day 10 an increase in both total and specific oxygen consumption rates was observed in both normal and diabetic mice at the same dose of T_3. This suggests that the young animals were no longer capable of compensating at the end stages of the experiment or that the metabolic response to T_3 required the full ten days of T_3 exposure to develop.

The increases seen in metabolic rates with T_3 treatment may be due to a variety of physiological responses including increases in thermogenesis and/or a change in the fuel source. Diabetic mice have been reported to have defects in thermogenesis which lead to increased metabolic efficiency (32). Thermogenesis is directly increased by thyroid hormones and indirectly through thyroid hormone potentiation of the thermogenic effects of epinephrine (33). Thus, the hyperthyroidism induced by the addition of exogenous T_3 to both the normal and diabetic mice may have lead to increased thermogenesis and thus, increased metabolic rates. Although the body temperatures of the mice were not measured, as the experiment progressed the mice receiving T_3 felt warmer to the touch compared to the vehicle treated control animals which supports the idea that these mice

were subjected to increased thermogenesis.

While the total metabolic response to T_3 likely accounts for most changes in oxygen consumption, oxygen consumption rates are also influenced by the fuel source used by the mice. Carbohydrates usually provide the major source of fuel for energy metabolism (21). However, as energy demands increase, the need for another fuel source becomes critical and lipids play a major role as an alternative source of fuel. Lipid catabolism, however, requires more oxygen per gram of fat than carbohydrate catabolism and therefore, could account for the increased oxygen consumption rates seen with T_3 treatment. A change to lipids as the fuel source could only occur if the concentrations of exogenous T_3 given in this experiment could overcome the proposed suppression of pituitary GH production and release caused by the hyperinsulinemia in diabetic mice (16). Subsequent production of GH would then enable the T_3-induced lipolytic response of adipocytes to occur.

To evaluate a potential shift in fuel source serum lipid profiles were evaluated. Nonfasting triglyceride levels were studied previously by Tuman and Doisy in diabetic mice and their lean littermates (7). The triglyceride concentrations of the diabetic mice were elevated at five weeks of age when compared to the normal mice, 120 ± 6 vs. 84 ± 4 mg/dl respectively. The triglyceride levels of the diabetic mice increased with age attaining a maximum level of 400 ± 91 mg/dl at 19 weeks of age while the normal mice had triglyceride levels that remained relatively constant throughout the experiment. Elevated serum concentrations of triglyceride were also observed in diabetic mice compared to their normal counterparts by Nishina et al (34). Triglyceride levels were found to be approximately four-fold greater in the diabetic mice than in the normal mice.

The triglyceride levels seen in the previous experiments correlate with the levels seen in the present study. It then became a question of whether lipid metabolism changed with T_3 treatment and if normal and diabetic animals responded in similar magnitudes to T_3.

The effect of thyroid hormone on the serum concentrations of triglyceride have been studied in the past and have been found to be quite variable (35). A majority of experiments indicate that hypothyroidism leads to increased levels of triglyceride while hyperthyroidism leads to decreased levels. Studies have suggested that the rate of hepatic synthesis and/or secretion of triglycerides is decreased in hyperthyroidism and increased in hypothyroidism. While this study attempted to evaluate serum lipid profiles the experimental design did not allow for the assessment of specific changes in triglyceride synthesis, secretion, and disposal. The normal animals in the present study had serum triglyceride levels that remained relatively constant at all doses of T_3 indicating no net change in circulating T_3 concentrations. The control diabetic animals, which exhibit hypertriglyceridemia, had elevated levels of triglycerides which may be the result of increased release of triglycerides from the livers (8) related to their effective "hypothyroid status" due to T_3 resistance. The increased level of serum triglycerides may also be due the diabetic animals decreased ability to clear triglycerides from the blood (8). The addition of exogenous T_3 to diabetic animals lead to a decrease in serum triglycerides and this may be due to T_3 suppression of triglyceride release from the liver leading to increased storage and/or an increased rate of removal from the blood by metabolically active tissues such as muscle. If the metabolic demand for triglycerides exceeds the synthesis and secretion by hepatocytes, the triglycerides will decrease in concentration in

the serum. These results suggest that T_3 influences serum triglyceride concentrations however, it is not possible to state whether it is synthetic or secretory control or uptake by metabolically active tissues.

Although the intent was to study all of the serum lipoproteins to evaluate lipid transport, this was not possible because the VLDL and LDL bands on the agarose gel were stained too lightly to quantitate. It was, however, possible to evaluate HDL and diabetic mice exhibited serum HDL concentrations that were six-fold greater than those observed in normal mice. Previous studies have also shown that C57BL/KsJ diabetic mice and genetically obese mice have elevated serum concentrations of HDL (34,36). It is interesting to note that the mice in both studies were subjected to an overnight fast prior to blood collection. The effect, if any, this had on the serum HDL concentrations is unknown. A direct comparison between the previous studies and this study cannot be made since the absolute concentrations of HDL were not determined.

The effects of altered thyroid status on plasma lipoproteins has been reviewed by Heimberg, Olubadewo, and Wilcox (35). The results are inconclusive as to the effect of thyroid hormone on serum HDL concentrations. It has been reported that hypothyroidism leads to elevated HDL cholesterol levels, however, other studies have shown HDL cholesterol levels to be decreased. Hyperthyroidism has been reported to lead to decreased serum HDL cholesterol levels. The results from the present study indicate that thyroid hormone resistance, which is functionally equivalent to hypothyroidism, leads to increased serum HDL concentrations. Hyperthyroidism resulting from administration of exogenous T_3, on the other hand, leads to decreased levels of HDL in both diabetic and

normal mice. It is clear that serum HDL concentrations are altered by T_3-sensitive responses, but the nature of these processes remain undefined.

Although metabolism of HDL has been the subject of many studies, the degradative processes and the tissues involved in HDL disposal remain unclear (37). The results from this study suggest that T_3 is an inhibitor of the release of HDL from the liver or an activator of the clearance of HDL from the serum. Normal animals given supraphysiological levels of T_3 exhibit a decrease in the serum HDL concentration (Figure 16) which would be consistent with inhibition of the release of HDL or an increase in the disposal rate. That normal animals do not exhibit any further decrease in serum HDL concentrations at the highest doses of T_3 may indicate that maximal inhibition occurs at the 100 ng T_3/g BW dose. If the elevated levels of serum HDL seen in the diabetic animals are due to a tissue insensitivity to the inhibitory effect of T_3 on the release of HDLs from the liver, T_3 resistance could lead to constitutive secretion of HDL from the liver thus leading to elevated serum HDL concentrations.

Since the half-life of HDL is 5 to 6 days (21) a two-fold increase in storage of HDLs should become apparent around day 5 of the experiment. If HDL secretion stopped, in 5-6 days the concentration of serum HDL would decrease to 50%, assuming there was no change in the rate of disposal. A 50% decrease in serum HDL would also be expected if the rate of secretion did not change but, the rate of disposal increased two-fold. During the ten days of observation there was enough time to see another 50% reduction in the serum HDL concentrations meaning that the HDL levels could theoretically decrease a total of 75%. The serum HDL concentrations of the diabetic mice in the present study

decreased by 60.49% at the 200 ng T_3/g BW dose which is less than what would be predicted from above. Unfortunately, HDL values in the fully stimulated (500 ng T_3/g BW) animals were not obtained. Thus, T_3 appears to play a role in either inhibiting hepatic HDL secretion or increasing degradation of serum HDL.

Interestingly, serum HDL concentrations in humans with NIDDM are stated to be significantly lower than serum HDL levels in normal individuals (38). However, it is imperative to note some critical differences between this study and those cited in the literature. First, studies of serum HDL levels have previously been studied in humans (38) while mice were used in this study. Although both are mammals and have many shared characteristics of NIDDM, it is possible that the difference in HDL profiles is due to a difference in the nature of the defect. In contrast, it has been noted that humans with NIDDM suffer from atherosclerotic vascular disease (38) while C57BL/KsJ db/db mice have decreased susceptibility to atherosclerosis (34). Since HDL is thought to play a role in reducing vascular disease via cholesterol scavenging (38) it may be that humans with NIDDM are more susceptible to atherosclerotic vascular disease than mice because of the decreased levels of serum HDL found in human subjects. Previous studies found an inverse correlation between HDL levels and the susceptibility to atherosclerosis in diabetic mice, the higher the serum HDL concentration the lower the risk of atherosclerosis (34,36). Third, the subjects discussed in the literature had been subjected to an overnight fast prior to blood collection (39) while the mice in the present study were not subjected to fasting. The importance of this difference seems to be negligible since previous studies with diabetic mice involved an overnight fast prior to blood collection (34,36) and these

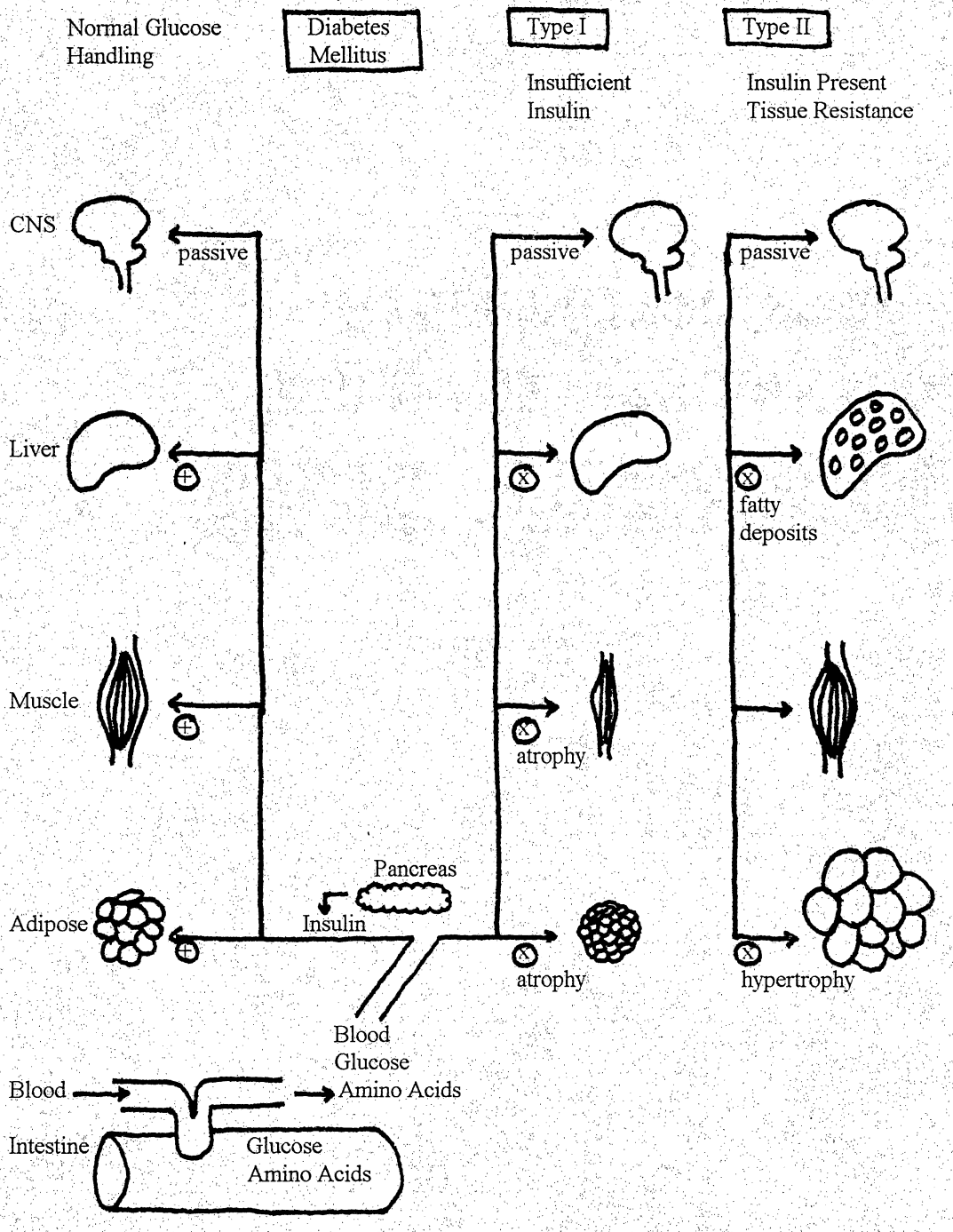
animals also had elevated serum concentrations of HDL. Thus, the serum concentrations of HDL in subjects with NIDDM appear to be dependent upon the particular form of NIDDM, humans have decreased while mice have increased levels of HDL.

Further studies will need to be conducted to ascertain the fuel source used by diabetic mice to sustain the elevated metabolism under T_3 stimulation. One way to ascertain the fuel source used would require a repetition of the treatment protocol used in this experiment with simultaneous measurement of both oxygen and carbon dioxide consumption rates. These would then be used to calculate the respiratory quotient (RQ) to determine if carbohydrates, proteins, or fats were being metabolized to provide the energy for the increased metabolism. Another important question which may be addressed is whether the increased levels of serum HDL seen in the diabetic mouse are due to T_3 resistance. One way to study the effects of T_3 on the release of HDL from the liver may be to inhibit the conversion of T_4 to T_3 by blocking the 5'-monodeiodinase with iopanoic acid (40). This would lead to a hypothyroid state and, if the normal action of T_3 is inhibition of production/release of HDL from the liver, would lead to increased serum HDL concentrations. This would be the first study that examines the influence of T_3 on HDL production.

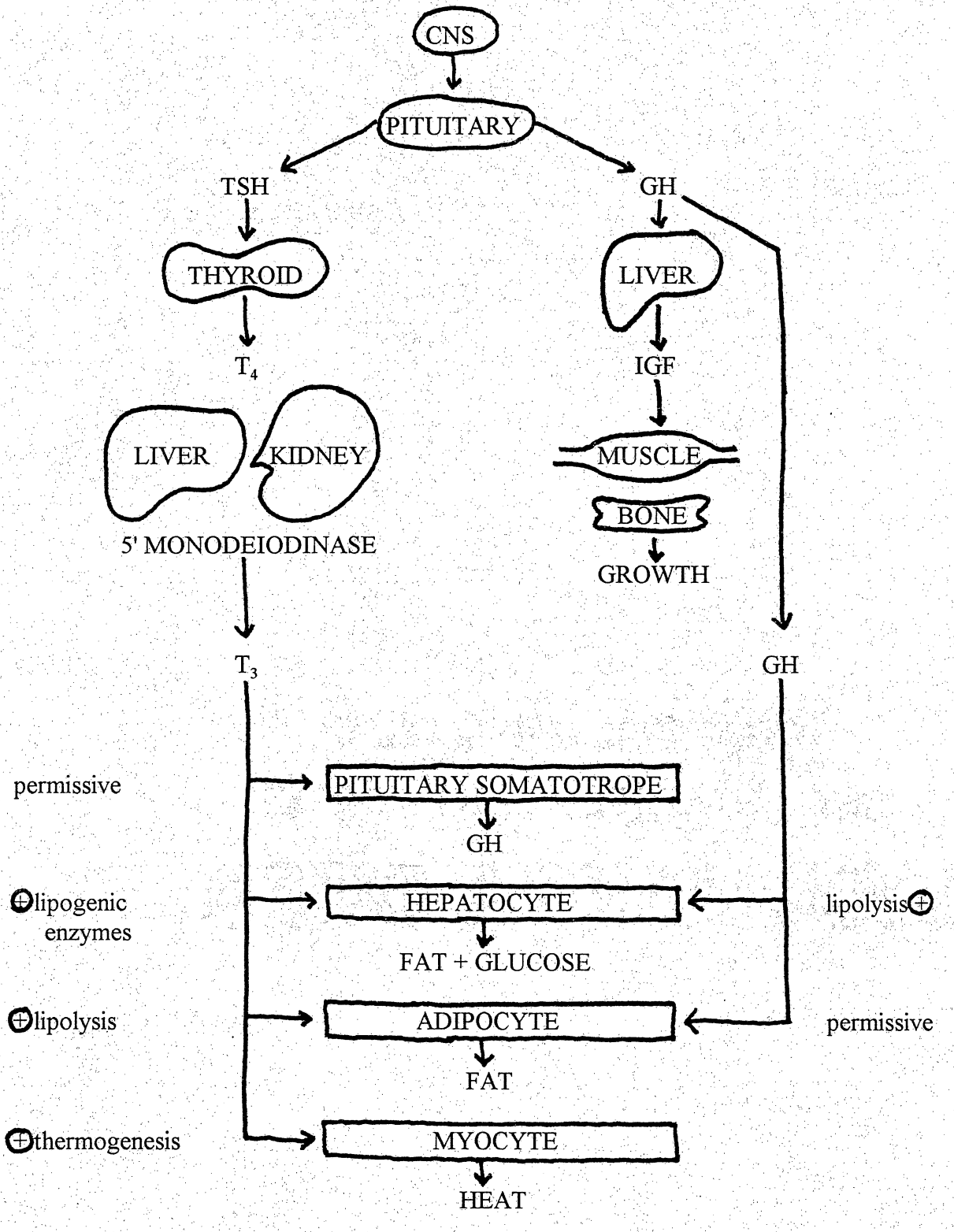
Previous studies examining the effects of T_3 treatment on body fat content in obese mice found that the body fat content decreased with increasing doses of T_3 (41). In the present study the percent body fat of normal mice remained relatively constant at all doses of T_3 except at 100 ng T_3/g BW where a small but significant increase was seen. The diabetic mice, on the other hand, exhibited decreases in percent body fat that were nearly

linearly dependent on the dose of T_3. The differences in responses between normal and diabetic mice may be due to diabetic mice being T_3 resistant. The thyroid hormone resistance may allow excessive fat deposition and the addition of the exogenous T_3 may enable diabetic mice to overcome this resistance. Thus, the normal balance of storage and release of fats in adipocytes may be restored.

This study has shown that diabetic mice are T_3 resistant and the degree of T_3 resistance in mice with NIDDM is age-dependent. The differences in specific oxygen consumption rates between normal and diabetic mice were shown not to be due to a metabolic scaling phenomenon related to body mass, but rather a T_3-responsive physiological mechanism, likely found in the lean body mass or inactive adipose tissues. The elevated levels of serum HDL in untreated diabetic mice and subsequent reductions with T_3 treatment also suggest that T_3 suppresses hepatic HDL production and/or release and that this process is similarly resistant to the action of T_3 in the C57BL/KsJ db/db diabetic mouse. This is the first report of T_3 regulation of HDL and extends the observations of T_3 resistance in the C57BL/KsJ diabetic mouse to include alterations in T_3 regulated lipid metabolism.



Appendix I. This diagram depicts the handling of glucose in normal individuals as well as in individuals with insulin dependent (Type I) and noninsulin dependent (Type II) diabetes mellitus.



Appendix II. This diagram depicts the normal actions of thyroid hormone and growth hormone.

Appendix III. Total oxygen consumption rates presented as mean ± SEM.

	NORMAL				DIABETIC			
	0 ng T_3/g BW	100 ng T_3/g BW	200 ng T_3/g BW	500 ng T_3/g BW	0 ng T_3/g BW	100 ng T_3/g BW	200 ng T_3/g BW	500 ng T_3/g BW
Day 1	1.109 ± 0.050	1.075 ± 0.077	1.022 ± 0.066	1.086 ± 0.022	1.117 ± 0.151	1.324 ± 0.177	1.288 ± 0.108	1.394 ± 0.092
Day 2	1.187 ± 0.040	1.232 ± 0.047	1.193 ± 0.059	1.254 ± 0.073	1.621 ± 0.036	1.546 ± 0.065	1.481 ± 0.037	1.557 ± 0.045
Day 3	1.355 ± 0.060	1.319 ± 0.012	1.296 ± 0.048	1.294 ± 0.062	1.691 ± 0.030	1.562 ± 0.076	1.537 ± 0.091	1.602 ± 0.062
Day 4	1.462 ± 0.078	1.523 ± 0.053	1.439 ± 0.036	1.602 ± 0.124	1.652 ± 0.085	1.669 ± 0.090	1.660 ± 0.049	1.728 ± 0.024
Day 5	1.526 ± 0.052	1.350 ± 0.033	1.504 ± 0.097	1.655 ± 0.078	1.789 ± 0.020	1.764 ± 0.083	1.702 ± 0.072	1.924 ± 0.086
Day 6	1.607 ± 0.080	1.593 ± 0.051	1.576 ± 0.067	1.786 ± 0.051	1.837 ± 0.048	1.898 ± 0.060	1.809 ± 0.081	2.050 ± 0.035
Day 7	1.330 ± 0.049	1.243 ± 0.030	1.336 ± 0.094	1.506 ± 0.076	1.613 ± 0.061	1.686 ± 0.073	1.761 ± 0.093	1.949 ± 0.063
Day 8	1.249 ± 0.109	1.453 ± 0.030	1.397 ± 0.027	1.604 ± 0.062	1.618 ± 0.046	1.711 ± 0.037	1.834 ± 0.049	1.912 ± 0.144
Day 9	1.243 ± 0.055	1.414 ± 0.062	1.285 ± 0.061	1.470 ± 0.073	1.546 ± 0.059	1.655 ± 0.042	1.702 ± 0.062	1.980 ± 0.041
Day 10	1.176 ± 0.061	1.235 ± 0.025	1.288 ± 0.015	1.400 ± 0.050	1.422 ± 0.050	1.789 ± 0.122	1.708 ± 0.068	1.968 ± 0.097

Appendix IV. Specific oxygen consumption rates presented as mean ± SEM.

	NORMAL				DIABETIC			
	0 ng T_3/g BW	100 ng T_3/g BW	200 ng T_3/g BW	500 ng T_3/g BW	0 ng T_3/g BW	100 ng T_3/g BW	200 ng T_3/g BW	500 ng T_3/g BW
Day 1	0.052 ± 0.0038	0.049 ± 0.0036	0.047 ± 0.0040	0.049 ± 0.0014	0.024 ± 0.0032	0.029 ± 0.0029	0.028 ± 0.0024	0.030 ± 0.0018
Day 2	0.055 ± 0.0023	0.056 ± 0.0028	0.054 ± 0.0037	0.055 ± 0.0019	0.035 ± 0.0010	0.034 ± 0.0009	0.032 ± 0.0008	0.033 ± 0.0012
Day 3	0.064 ± 0.0034	0.060 ± 0.0016	0.060 ± 0.0035	0.058 ± 0.0012	0.037 ± 0.0009	0.035 ± 0.0014	0.033 ± 0.0017	0.035 ± 0.0022
Day 4	0.069 ± 0.0044	0.071 ± 0.0025	0.067 ± 0.0031	0.073 ± 0.0037	0.035 ± 0.0017	0.037 ± 0.0011	0.036 ± 0.0012	0.038 ± 0.0014
Day 5	0.073 ± 0.0037	0.064 ± 0.0020	0.070 ± 0.0058	0.076 ± 0.0032	0.038 ± 0.0006	0.040 ± 0.0018	0.038 ± 0.0009	0.043 ± 0.0007
Day 6	0.075 ± 0.0050	0.074 ± 0.0027	0.073 ± 0.0038	0.082 ± 0.0031	0.039 ± 0.0009	0.044 ± 0.0026	0.041 ± 0.0009	0.047 ± 0.0013
Day 7	0.063 ± 0.0037	0.057 ± 0.0011	0.062 ± 0.0046	0.069 ± 0.0037	0.034 ± 0.0012	0.039 ± 0.0024	0.040 ± 0.0013	0.046 ± 0.0032
Day 8	0.059 ± 0.0044	0.066 ± 0.0015	0.065 ± 0.0016	0.073 ± 0.0041	0.034 ± 0.0011	0.040 ± 0.0018	0.043 ± 0.0012	0.046 ± 0.0031
Day 9	0.058 ± 0.0025	0.064 ± 0.0029	0.059 ± 0.0030	0.065 ± 0.0033	0.033 ± 0.0011	0.040 ± 0.0012	0.040 ± 0.0015	0.048 ± 0.0009
Day 10	0.055 ± 0.0038	0.055 ± 0.0012	0.059 ± 0.0011	0.062 ± 0.0018	0.030 ± 0.0009	0.044 ± 0.0038	0.041 ± 0.0009	0.050 ± 0.0026

Appendix V. Serum triglyceride concentrations presented as mean ± SEM.

(mg/dL)

	0 ng T_3/g BW	100 ng T_3/g BW	200 ng T_3/g BW	500 ng T_3/g BW
NORMAL	79.55 ± 3.37	76.85 ± 4.13	107.78 ± 6.61	86.93 ± 6.20
DIABETIC	365.06 ± 20.60	225.43 ± 17.50	258.73 ± 33.28	217.05 ± 13.50

Appendix VI. Serum HDL concentrations presented as mean ± SEM.

(O. D.)

	0 ng T_3/g BW	100 ng T_3/g BW	200 ng T_3/g BW
NORMAL	0.386 ± 0.116	0.144 ± 0.034	0.220 ± 0.081
DIABETIC	1.544 ± 0.053	1.134 ± 0.062	0.610 ± 0.056

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